Taketo Nakano* & Shinji Handa**: Two species of corticolous *Chlamydomonas* new to Japan***

中野武登*・半田信司**: 樹皮着生クラミドモナス属の日本新産2種

The genus Chlamydomonas is comprised of a group of flagellated unicellular algae in the Chlorophyceae. About 500 species of the genus have been described in the world (Ettl 1976), but only the following nine species have been reported from Japan: Chlamydomonas angulosa Dill, C. basistellata Pascher, C. moewusii Gerloff, C. monticola S. Watanabe, C. nivalis (Bauer) Wille var. kobayasii Fukushima, C. pertusa Chodat, C. pyrenoidosa Schiller, C. reinhardtii Dangeard and C. umbonata Pascher (Hirose & Yamagishi 1977, Watanabe 1983). These species were known to occur usually in freshwater and soil, and rarely in seawater. In the course of a floristic study of corticolous algae in Hiroshima Prefecture, we isolated and identified two species of Chlamydomonas from tree bark, and found that these species were newly recorded as corticolous algae and are new to Japanese flora.

This paper provides descriptions, illustrations and some taxonomical notes on these two species.

Material and methods A bark sample (Handa-184 in HIRO) was collected from a tree of *Cryptomeria japonica* (L.f.) D. Don by striping the trunk with knife in April, 1981, at Migihira, Ogauchi, Asa-cho, Hiroshima-city, at an altitude of approximately 300 m. The sampling portion was about 1 m high above the ground on the east side of the trunk. On the surface of the bark, green algal colonies were observed macroscopically. In the laboratory, attached materials including algae were gently scraped from the surface of the bark, and they were spread on agar plates containing 1N BBM as modified by Bischoff & Bold (1963) under cool white fluorescent lamps on a cycle of 12 h light and 12 h darkness at approximately 20° C. The irradiance was about $20 \, \mu$ E·m⁻²·s⁻¹.

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Axenic cultures were obtained by the method of Wiedeman et al. (1964), and these axenic cultures were observed with the light microscope.

1) Chlamydomonas parvula Gerloff, Arch. f. Protistenk. 94: 380, 463, f. 36, 1940. Etll, Beih. z. Nova Hedwigia 49: 427-428, pl. 71, f. 1, 1976. (Figs. 1-6)

Cells ellipsoidal to cylindrical, with rotund ends, $4.5\text{-}6.5\,\mu\text{m}$ wide, $9.0\text{-}12.0\,\mu\text{m}$ long. Cell wall thin and smooth without papilla. Flagella two, equal to or sometimes slightly shorter than the cell length. Chloroplast urn-shaped, encircling 3/4-4/5 of the cell lumen, rarely with a few incisions (*Chlamydella* type, Ettl 1976). Pyrenoid lateral or rarely central, single or rarely double, surrounded by two starch grains, $1.5\text{-}2.5\,\mu\text{m}$ in diameter. Stigma situated at the median to slightly anterior portion of the cell. Two contractile vacuoles present at the anterior end, and a nucleus at the posterior. Asexual reproduction by forming of 2-8 daughter cells. Sexual reproduction not observed.

Habitat: Growing in soil and on bark.

Geographical distribution: Yugoslavia and Japan.

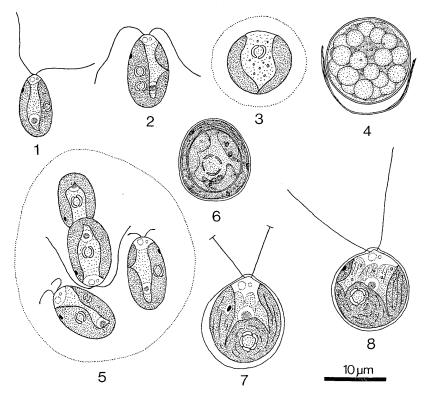
Specimen examined: Hiroshima-shi, Asa-cho, Ogauchi, Migihira (Handa-184).

This species is newly found as a corticolous alga and newly reported from Japan.

Our alga agrees well with the description by Gerloff (1940) and Ettl (1976) except for the number of pyrenoids and the number of starch grains surrounding the pyrenoid. According to the descriptions, this alga has one pyrenoid surrounded by many starch grains, whereas our specimen has usually one pyrenoid and rarely has two pyrenoids, which are surrounded by two starch grains (Fig. 2).

When motility ceased, gelatinous matrices were deposited around the cell which became broad-ellipsoidal to spherical in shape (Fig. 3). Cells attained a diameter of $12.0\,\mu\text{m}$. Akinetes were also observed in old cultures (Fig. 4). The akinete cells are $14.0\,\mu\text{m}$ in diameter, with slightly thickened cell walls. Inner structure of these cells could not be observed in detail, because many oil droplets were accumulated in the cell lumen. Asexual reproduction occurred in these nonmotile stages. Two to eight daughter cells were formed in a mother cell. After the daughter cells became free from the mother cell wall, they were gradually separated from each other and discharged (Fig. 5).

In this study, the process of sexual reproduction could not be observed, but we found some zygote-like cells (Fig. 6). They were spherical and had thick-



Figs. 1-8. Chlamydomonas parvula Gerloff. 1, 2. Motile cells. 3. Nonmotile cell enveloped by gelatinous matrix. 4. Akinetes. 5. Four daughter cells enveloped by gelatinous matrix.
6. Zygote-like cell. Figs. 7, 8. Chlamydomonas pila Ettl. Motile cells.

ened cell wall in which a chloroplast with one pyrenoid and two stigmata were observed. The cell wall was brown in color. Further development of these cells could not be observed.

This species is similar to *C. peterfii*, but it can be readily identified by its cylindrical cell and posterior nucleus and arrangement of starch grains surrounding pyrenoid.

2) Chlamydomonas pila Ettl, Arch. f. Protistenk. 108: 289, f. 10, 1965. Ettl, Beih. z. Nova Hedwigia 49: 388, pl. 51, 1976. (Figs. 7, 8)

Cells broadly ellipsoidal to spherical, 5.0-11.0 μ m wide, 6.5-14.0 μ m long. Hull usually thin or rarely thick. Papilla low and broad, without definite edge,

slightly visible at the anterior end. Flagella two, about 1.5 times the cell length. Chloroplast generally cup-shaped with some deep incisions at the margin and with many grooves (Euchlamydomonas type, Ettl 1976). Pyrenoid spherical or broadly ellipsoidal, surrounded by many starch grains, about 3.5 μm in diameter, situated at the posterior to the slightly median portion of the cell. Stigma ellipsoidal to globular, situated at the median to the slightly anterior and the nucleus at the median to the slightly anterior part. Two contractile vacuoles present at the anterior end. Asexual and sexual reproduction not observed.

Habitat: Growing in soil and on bark.

Geographical distribution: Czechoslovakia and Japan.

Specimen examined: Hiroshima-shi, Asa-cho, Ogauchi, Migihira (Handa-184).

This is newly reported in Japan and newly recorded as a corticolous alga.

Japanese specimen agrees well with the description by Ettl (1965, 1976) except for the presence of a hull. Although Ettl (1976) reported that it always has thin hull, the hull of our specimen is usually thin but rarely thick. According to the original description, this species makes asexual reproduction (formation of daughter cells), but we could not find it in our specimen. Sexual reproduction has not been known until now.

This species is similar to *C. amici-mei* in general morphology, but the latter species differs in having a smaller stigma and lacking papilla.

So far as we know, there are several papers published concerning *Chlamydomonas* found on the bark of trees (Cox & Hightower 1972, Edwards 1968, Graham et al. 1981, Green 1969). Unfortunately, however, all of corticolous *Chlamydomonas* were reported without specific names. Therefore, the present paper is the first to deal with the names at species level for the corticolous *Chlamydomonas*.

We wish to express our sincere thanks to Professor Z. Iwatsuki of Hiroshima University for critically reading the manuscript and Professor P. A. Archibald of Slippery Rock University for her comments on the manuscript.

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広島市安佐町小河内右平のスギの樹皮から分離したクラミドモナス属の2種について 形態,分類学的特徴,他種との区別点等について報告した。クラミドモナス属は2本の 等長の鞭毛を持つ単細胞性緑藻類の一群であり、淡水(稀に海水)、土壌中等に広く分 布する。また、樹皮着生藻類としても報告があるが、属レベルでの報告のみであって、 種は明らかにされていなかった。本属については現在までに約500種が記載されている が、日本産のものは9種が報告されているに過ぎない。今回報告した以下の2種はいず れも日本新産であり、かつ樹皮着生藻類としては初めての記録である。1) Chlamydomonas parvula: 細胞は楕円体または円筒形で、パピラを持たず、核は細胞後部に位置 する。娘細胞、アキネート、接合子らしい細胞が観察された。2) C. pila: 細胞は広楕 円体又は球体、平坦で短いパピラを持ち、葉緑体はカップ状で、深い切れ込みを持つ。 厚い被膜を持つ細胞が稀に観察されたが、この点に関しては更に詳細な検討を要する。